РАЗДЕЛ I. ДИСФУНКЦИЯ ЭНДОТЕЛИЯ КРОВЕНОСНЫХ СОСУДОВ: ОБЩИЕ МЕДИКО-БИОЛОГИЧЕСКИЕ ВОПРОСЫ

HYPOXIC CONDITIONING SUPPRESSES CYTOTOXIC NITRIC OXIDE PRODUCTION BY ENDOTHELIUM UPON REPERFUSION FOLLOWING ACUTE MYOCARDIAL ISCHEMIA

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There is increasing evidence that hypoxic conditioning, in particular intermittent and repetitive exposure to moderate hypoxia, induces adaptive responses that protect the heart from ischemic insults.[1] A number of mechanisms have been proposed to account for the cardioprotective responses to hypoxia stress. These mechanisms include 1) activation of ATP-sensitive K^+ channels in cellular[2,3] and mitochondrial[4-6] membranes, 2) production of cardioprotective proteins including glycolytic enzymes[7,8] and heat-shock proteins,[9-12] 3) release of erythropoietin,[13] 4) increased production of NO by enhanced inducible nitric oxide synthase,[14-16] 5) angiogenesis,[17] 6) attenuation of apoptosis,[18] and enhancement of antioxidative defenses.[19-21] Although there is evidence that hypoxia-induced increase in NO is cardioprotective,[22,23] there is also evidence that excessive NO might exacerbate myocardial damage.[24,25]

In the first study of hypoxia induced cardioprotection in a large animal, we found remarkable attenuation of myocardial infarct size and absence of lethal arrhythmias in hypoxia-conditioned dogs.[26] As shown in Figure 1, dogs that completed a program of intermittent, moderate hypoxia (9.5 -10.0% FIO_2, 5-10 min/cycle, 5-8 cycles/d with intervening 4 min normoxia for 20 d) had negligible myocardial infarction following 1 hr occlusion and 5 hr reperfusion of the left anterior descending coronary artery (LAD). Hearts of untreated control dogs had large infarctions, and the majority of these untreated animals developed ventricular tachycardia or ventricular fibrillation. Collateral blood flow to the ischemic territory did not differ among the groups, so cardioprotection by intermittent hypoxia was not due to collateral angiogenesis.

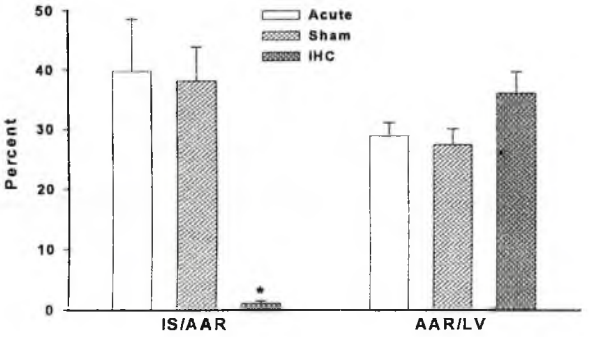

Figure 1. Intermittent, normobaric hypoxia conditioning (IHC) prevents myocardial infarction. Masses of myocardial infarct (infarct size; IS), ischemic area at risk (AAR), and left ventricular free wall + septum (LV) were measured after 1 hour occlusion of the left anterior descending coronary artery and 5 h reperfusion. Values are means ± SEM from 7 acute non-conditioned dogs (open bars), 12 dogs hypoxia-conditioned for 20 consecutive days (IHC; cross-hatched bars), and 10 dogs sham-conditioned with normoxic air for 20 consecutive days (hatched bars). *P < 0.001 *vs.* both non-hypoxic groups.

Whereas increased myocardial NO may be a beneficial result of hypoxia conditioning, excessive release of NO by endothelium during reperfusion may be deleterious. Thus, we hypothesized that hypoxia conditioning suppresses myocardial formation of excessive NO by the endothelial NO synthase isoform (eNOS) upon coronary artery reperfusion. In the absence of hypoxic conditioning, excessive NO would react with superoxide to produce cytotoxic peroxynitrite and lead to myocardial injury.[27]

Materials and Methods

Mongrel dogs were conditioned by a 20 d program of intermittent normobaric hypoxia as described above,[26] and compared with non-conditioned control dogs. One day after the conditioning program was completed, myocardium was sampled to measure left ventricular NO synthase (NOS) activity (colorimetric assay) and eNOS content (Western blot). In other anesthetized dogs, myocardial nitrite release, an index of NO formation,[28,29] was measured in coronary venous blood during reperfusion following 1 h occlusion of the left anterior descending coronary artery (LAD).

Results

Figure 2 shows that hypoxic conditioning lowered left ventricular total NOS activity 45%, from 92 ± 8 to 43 ± 6 mU/g protein (P<0.01).

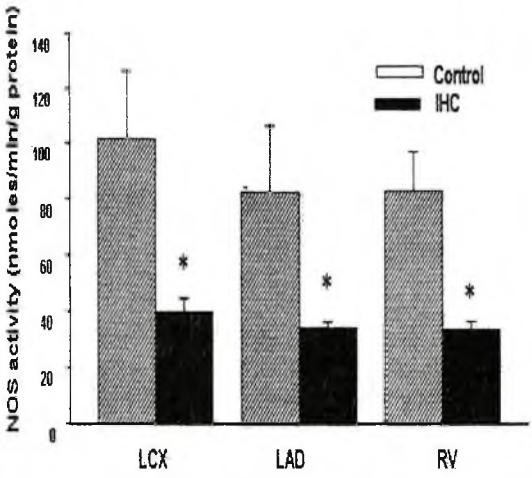

Figure 2. Myocardial nitric oxide synthase (NOS) activity. Total NOS activity was measured in left ventricular myocardium from the left circumflex (LCX) and left anterior descending (LAD) coronary artery perfusion territories, and in right ventricular myocardium (RV) Values are means ± SEM from 7 non-conditioned (control) and 6 hypoxia-conditioned (IHC) dogs. *P < 0.05 *vs.* control.

Consistent with these data is the finding that nitrite release into coronary venous blood upon reperfusion was sharply reduced in hypoxic conditioned dogs.

Figure 3 illustrates that peak nitrite release immediately after reperfusion was greatly reduced in the hypoxia conditioned dogs,

and Figure 4A shows that significantly less total nitrite was released during the first 5 min of reperfusion in these dogs. If coronary flow were reduced during reperfusion in the hypoxia-conditioned dogs, reduced endothelial shear stress might lessen NO production and decrease nitrite release. Figure 4B shows that peak flow in the reperfused LAD was similar in control and hypoxia conditioned dogs, suggesting that NO does not play an essential role in early reperfusion hyperemia; moreover, lower nitrite release in the hypoxia-conditioned dogs was not due to decreased flow-dependent shear stress. It is interesting that between 5 and 10 min of reperfusion, both flow and nitrite release were greater in the control hearts.

Figure 5 shows a Western blot for analysis of eNOS in samples of LAD perfused myocardium from three hypoxia-conditioned and four control dogs. It is apparent that eNOS content was reduced in the hypoxia-conditioned myocardium.

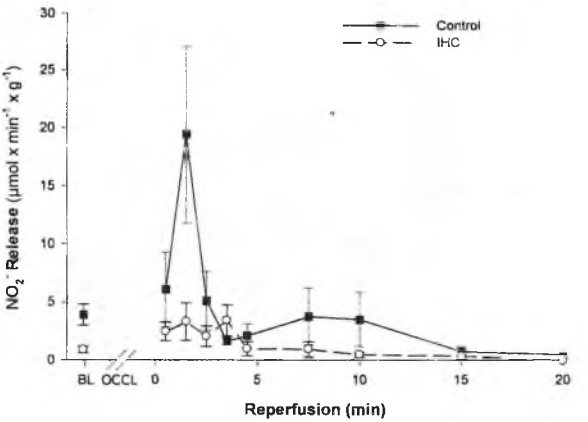

Figure 3. Left ventricular myocardial nitrite (NO_2^-) release. NO_2^- was measured in plasma sampled simultaneously from the aorta and interventricular coronary vein during pre-ischemic baseline (BL) and reperfusion following 60 min LAD occlusion (OCCL). Means ± SEM from 5 experiments per group.

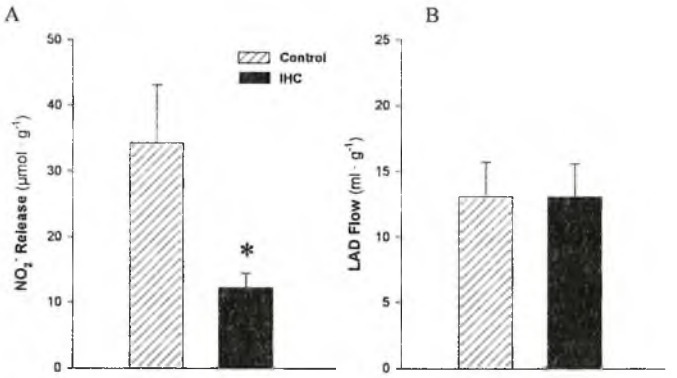

Figure 4. Cumulative myocardial NO_2^- release (panel A) and LAD flow (panel B) during the first 5 min of LAD reperfusion. Means ± SEM from 5 experiments per group. *$P < 0.05$ vs. control.

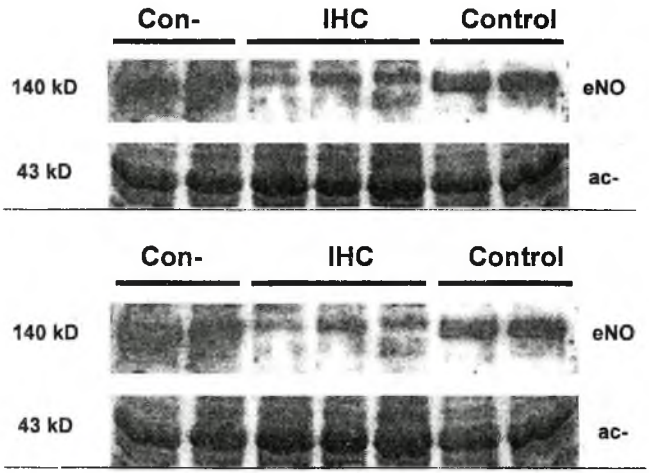

Figure 5. Western blot of eNOS in left ventricular myocardium. Total protein (50 μg) from homogenates of left ventricular myocardium was electrophoretically separated, transferred to nylon membrane, and probed with eNOS-specific antibody. Actin (Coomassie blue-stained gel) served as loading control.

Discussion

Hypoxia conditioning protects myocardium from ischemic injury. It is likely that multiple mechanisms contribute to this protection. Blocking NO synthesis can exacerbate myocardial ischemic damage,[22] so NO is an important cardioprotective agent. However, moderate reductions in NO synthesis are cardioprotective,[30,31] suggesting that beyond an optimal concentration, excessive NO may contribute to ischemic damage. This is especially likely in the setting of coronary artery reperfusion, where endothelial release of NO would be accelerated to the extent that cytotoxic peroxide and other NO derivates might be formed in the presence of elevated superoxide.[27]

The present findings demonstrate that hypoxic conditioning of dogs reduces myocardial NOS activity and release of nitrite into coronary venous blood at reperfusion. Since hypoxia is expected to elevate iNOS,[14-16] the present finding of reduced total NOS activity could be explained if eNOS were concurrently reduced. Our Western blot results demonstrate that this is the case. Thus, we conclude that abruptly reperfused coronary endothelium releases excessive NO, so cytotoxic peroxynitrite and other degradation products of NO are formed. Hypoxia conditioning attenuates eNOS activity, prevents excessive NO upon reperfusion and, thus, protects myocardium from ischemia-reperfusion injury.

Acknowledgements

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NO-ЗАВИСИМЫЕ МЕХАНИЗМЫ ФОРМИРОВАНИЯ КИСЛОРОДСВЯЗУЮЩИХ СВОЙСТВ ГЕМОГЛОБИНА

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В большинстве исследований, термин "дисфункция эндотелия" используется как синоним нарушений эндотелиального метаболизма L-аргинин-NO системы, так как монооксид азота (NO) является сигнальным агентом, участвующим в опосредовании его функций и основное его количество, образуемого в организме, осуществляется в эндотелии. Снижение синтеза NO может быть вызвано: отсутствием исходного субстрата - L-аргинина, отсутствием кофакторов, сниженной экспрессией фермента и повышением уровня эндогенных ингибиторов фермента (например, асимметрично диметилированных производных аргинина). Кроме того, снижение антиоксидантной способности организма также может вести к зависимой от NO дисфункции эндотелия, так как снижение его эффектов может вызываться усиленной его инактивацией в результате повышенной выработки кислородных радикалов.

В последние годы проблема изучения физиологических эффектов NO приобрела новый аспект, а именно его взаимодействие с различными компонентами крови, и в частности, с гемоглобином. Предложена теория сосудистого «сохранения» и транспорта NO гемоглобином. В литературе широко обсуждались различные стороны этого вопроса, что получило отражение в ряде публикаций с весьма интригующими заголовками: «Гемоглобин: транспортер NO, инактиватор NO или ни то ни другое?» [Hobbs A.J. et al., 2002]; «Реакции азота с гемоглобином: взгляд через шторм SNO» [Gladwin M.T. et al., 2003];